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CRYOGENIC MOUNTING, ARCHIVING AND TRANSPORTATION OF BIOLOGICAL MACROMOLECULAR CRYSTALS.

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Cryogenic data collection for macromolecular crystals is increasing in popularity as a means of improving data quality. The advantages include the postponement of radiation damage, elimination of errors arising from inter-crystal scaling and merging, reduced thermal motion, enhanced contrast in electron density maps, and a possible reduction in conformational disorder.

Crystals are generally cooled after modification of the aqueous layer surrounding the surface of the crystal, either by some antifreeze (cryoprotectant) or oil. When a crystal is sufficiently robust that it can survive cooling by being placed directly in a cold gas stream, that should be the cooling method of choice. Often, however, successful crystal cooling requires that the cooling operation be as rapid as possible. A currently popular myth dictates that the fastest transition between room and cryogenic temperatures is achieved by dunking the crystal in liquid propane. In reality, dunking in liquid nitrogen provides faster cooling and is not prone to the problems of bubble formation that are known to plague larger samples, as is seen in electron microscopy. Safety concerns surrounding the use of open containers of liquid propane in close proximity to electrical equipment are also not an issue with liquid nitrogen.

Tools and techniques are presented for the easy manipulation, mounting, dismounting, archiving and transportation of sensitive crystals based on immersion in liquid nitrogen. These techniques have been in routine operation in our laboratories for several years now. They are simple to learn and above all they are reproducible and safe under all circumstances. The temperature history of a crystal during the full procedure of mounting, dismounting, transfer to a storage dewar and remounting has been measured and it is shown that it never rises above the temperature of the warmest component of the whole cryogenic system, namely the cold gas stream. These procedures now form the preferred modus operandi at the Stanford Synchrotron Radiation Laboratory (SSRL).

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